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(71) Applicant *(for all designated States except US)*: **EUROPEAN MOLECULAR BIOLOGY LABORATORY** [DE/DE]; Postfach 102209, 69012 Heidelberg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DE MARCO, Ario** [IT/DE]; Am Waldrand 10, 69126 Heidelberg (DE). **GEERLOF, Arie** [NE/DE]; Schelklystr. 74-6, 69126 Heidelberg (DE). **BUKAU, Bernd** [DE/DE]; Albert Ueberle-Strasse 6, 69120 Heidelberg (DE). **DEUERLING, Elke** [DE/DE]; Am Venusberg 22, 79111 Freiburg (DE).

(74) Agents: **GOODFELLOW, Hugh, Robin** et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

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(54) Title: RECOMBINANT PROTEIN EXPRESSION

(57) Abstract: There are provided methods for the expression of a recombinant protein of interest, said methods comprising, in additional to various additional steps: a) culturing a host cell which expresses: i) one or more genes encoding the recombinant protein(s) of interest; ii) at least two genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GRoES, Dnak, DnaJ, GRpe, ClpB and their homologs (for example, Hsp104, Ydj1 and Ssal in yeast); under conditions suitable for protein expression; and separating said recombinant protein of interest from the host cell culture. Also provided are methods for increasing the degree of refolding of a recombinant protein of interest by ading a composition containing a chaperone protein to a preparation of the recombinant protein of interest in vitro.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 02/00299

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P21/00 C12N15/67 A61K48/00 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 885 967 A (HSP KENKYUSHO KK) 23 December 1998 (1998-12-23) column 3, line 41 - line 55; claims 1-12 column 4, line 7-55 column 19, line 11 - line 13	1-5, 7-12,20, 21,25
X	WO 00 71723 A (BUKAU BERND ;ROCHE DIAGNOSTICS GMBH (DE); GOLOUBINOFF PIERRE (IL)) 30 November 2000 (2000-11-30)	1-5, 7-11, 20-25,34
Y	figures 4,9; example 6	6,15-18, 22-25,35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2003	27.08.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mabit, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOGK A ET AL: "Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 18, no. 24, 15 December 1999 (1999-12-15), pages 6934-6949, XP002148774 ISSN: 0261-4189 cited in the application page 6942, column 1, line 1 -page 6943, column 1; figure 6 ---	1-5,7, 10,11, 18, 20-25, 34,35
X	CARRIO M M ET AL: "Protein aggregation as bacterial inclusion bodies is reversible" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 489, no. 1, 26 January 2001 (2001-01-26), pages 29-33, XP004239392 ISSN: 0014-5793 cited in the application	1-5, 12-14, 20,22, 25,26
Y	page 30, column 1, paragraphs IN,VIVO,REFOLDING,OF,IB,PROTEIN ---	15-17
X	AMREIN KURT E ET AL: "Purification and characterization of recombinant human p50-csk protein-tyrosine kinase from an Escherichia coli expression system overproducing the bacterial chaperones GroES and GroEL." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 92, no. 4, 1995, pages 1048-1052, XP002241051 1995 ISSN: 0027-8424 cited in the application page 1049, column 2, paragraph RESULTS -page 1050, column 1, line 2; figure 1 ---	1-5,7, 10, 20-22, 24-26
X	THOMAS JEFFREY G ET AL: "ClpB and HtpG facilitate de novo protein folding in stressed Escherichia coli cells." MOLECULAR MICROBIOLOGY, vol. 36, no. 6, June 2000 (2000-06), pages 1360-1370, XP002241052 ISSN: 0950-382X page 1361, column 2, line 18-22 page 1361, column 2, line 44-47 page 1363, column 2, line 17-19 ---	1-5,7, 10,11, 18,20, 21,24,25

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C.(Continuation) DOCUMENTS CONSIDERED TO [redacted] ELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEN-ZVI ANAT PERES ET AL: "Review: Mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones." JOURNAL OF STRUCTURAL BIOLOGY, vol. 135, no. 2, August 2001 (2001-08), pages 84-93, XP002241053 ISSN: 1047-8477 page 86, column 2, paragraph 2 page 85, column 1, line 2-6 page 87, column 2, paragraph 2 -page 88, column 1 figures 4,5 ---	1-5,7, 10,11, 18-26, 34,35
Y	VEINGER LEA ET AL: "The small heat-shock protein IbpB from Escherichia coli stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 18, 1 May 1998 (1998-05-01), pages 11032-11037, XP002241887 ISSN: 0021-9258 page 11032, column 2, paragraph 4 figure 6 -----	6,17,18, 22-25,35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00299

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-26 and claims 34-35 partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26 and claims 34-35 partially

1.1. Claims: 1-5, and claims 7-14, 18-26, and 34-35 partially

A method for the expression of a recombinant protein of interest, said method comprising: a) culturing a host cell which expresses: i) one or more genes encoding the recombinant protein of interest; ii) at least two genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs under conditions suitable for protein expression and b) separating said recombinant protein of interest from the host cell culture

1.2. Claims: claim 6 and claims 7-14, 18-26, 34-35 partially

A method for the expression of a recombinant protein of interest, said method comprising: a) culturing a host cell which expresses: i) one or more genes encoding the recombinant protein of interest; ii) one or more genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs and iii) one or more genes encoding proteins selected from the group consisiting of the small heatshock proteins of the IbpA family and/or the IbpB family and /or their homologs b) separating said recombinant protein of interest from the host cell culture

1.3. Claims: 15 and claims 17-26, 34-35 partially

A method for the expression of a recombinant protein of interest, said method comprising: a) culturing a host cell which expresses: i) one or more genes encoding the recombinant protein of interest; ii) at least two genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs under conditions suitable for protein expression and b) Imposing a block in protein synthesis once a desired level of recombinant protein of interest has accumulated and, c)separating said recombinant protein of interest from the host cell culture

1.4. Claims: claims 16 and claims 17-26, 34-35 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for the expression of a recombinant protein of interest, said method comprising: a) culturing a host cell which expresses: i) one or more genes encoding the recombinant protein of interest; ii) at least two genes encoding proteins selected from the group consisting of the chaperone proteins GroEL, GroES, DnaK, DnaJ, GrpE, ClpB and their homologs under conditions suitable for protein expression and b) Imposing a reduction in gene transcription once a desired level of recombinant protein of interest has accumulated andc)separating said recombinant protein of interest from the host cell culture

2. Claims: 27-33 and claims 34-35 partially

A method for increasing the degree of refolding of a recombinant protein of interest, said method comprising adding a composition containing a chaperone protein to a preparation of the recombinant protein of interest in vitro.

3. Claims: 36-40

The use of one or more proteins selected from the group consisting of the chaperone proteins GroEl, GroES, DnaK, DnaJ, GrpE, ClpB, and their homologs, and one or more genes encoding proteins selected from the group consisting of the small heatshock proteins of the IbpA family and/or the IbpB family and /or their homologs, in the manufacture of a medicament for the treatment of disease in which the presence of aggregated proteins are implicated.
A method of treating a patient suffering from a disease in which the presence of aggregated proteins is implicated, comprising administarting one or more of said genes or proteins

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 02/00299

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0885967	A	23-12-1998	JP	3344618 B2	11-11-2002
			JP	11009274 A	19-01-1999
			CA	2235468 A1	20-12-1998
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